REMARKS/ARGUMENTS

Claims 5-6 and 21-35 are currently pending in the application. Claims 5-6 and 21-29 stand rejected. New claims 30-35 have been added. The new claims are directed to compounds already encompassed by genus claim 5 and compound 65 identified in the specification. Claims 5, 23, and 25-27 have been amended.

Applicants appreciate the comments and suggestions of the Examiner in an interview conducted on April 18, 2006. In accordance with the partial agreement reached (and summarized in the interview summary), Applicants submit this response to the outstanding Office Action, which provides additional information regarding the unexpected properties of the claimed compounds. Applicants believe that in light of the arguments and information submitted herewith, the claims as amended are allowable, and requests the issuance of a timely notice of allowance.

In the Specification

By this paper, various typographical errors have been corrected in the specification. Substitute pages 68 and 68A have been entered to provide legible chemical drawings noted as missing or illegible on page 36 of the published application (US 2004/0039014). In addition, substitute pages 72 and 72A have been entered to correct a typographical error in the chemical structure for 4-dimethylaminophenyl-diphenylphosphine on page 37, which included a fluoro atom ("F") instead of a phosphorous atom ("P"). Support for the correction can be found in paragraphs 199 and 202 of the specification.

No new matter has been added by these corrections and substitutions.

35 U.S.C. § 103(a) Obviousness Rejection of Claims 5-6 and 21-24

Claims 5-6 and 21-24 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Mueller et al. (WO 96/40097, herein after "Mueller") in view of Skolnick et al. (Pharmacopsychiatry, Abstract, 1996 January, 29:1, 23-6, herein after "Skolnick").

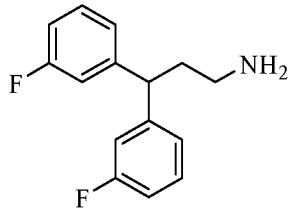
As briefly discussed in the interview, Applicants traverse the obviousness rejection because the prior art relied upon for the rejection teaches away from the claimed invention. Further, Applicants provide evidence herewith to show unexpected results and properties of the claimed compounds.

M.P.E.P. 706.02(j) sets forth the standard for a Section 103(a) rejection:

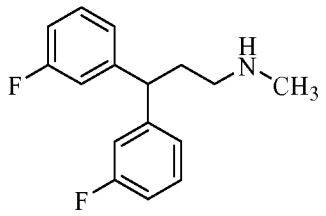
> To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). Also,

A *prima facie* case of obviousness may be rebutted by showing that the art teaches away from the claimed invention (*see* M.P.E.P. 2141.02 *citing W. L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540 (Fed. Cir. 1983)) or by evidence of unexpected results (*see* M.P.E.P. 2144.09 *citing In re Papesch*, 315 F.2d 381 (CCPA 1963)).

The Office Action states that Mueller discloses compounds 20 and 60 (pages 62 and 64)



WO 96/40097 (Compound 20)



WO 96/40097 (Compound 60)

as NMDA receptor antagonists useful for treating neurological diseases including epilepsy, Alzheimer's, Parkinson's, and Huntington's diseases. The Office Action also states that Skolnick teaches that NMDA antagonists mimic the effects of clinically effective antidepressants and that the NMDA receptor is involved in the pathophysiology of depression (Skolnick abstract). The Office Action concludes that in light of the teaching of Skolnick, it would be obvious to treat depression using compound 60 from Mueller.

Applicants traverse the rejection for the following reasons: first, Skolnick as a whole teaches away from the claimed invention, and second, the claimed methods of treating depression with the recited compositions impart unexpectedly improved properties or properties not present in the prior art. For these reasons, Applicants respectfully submit that the obviousness rejection should be withdrawn and the claims be allowed.

A. <u>The prior art relied upon for the rejection teaches away from the claimed invention.</u>

Applicants traverse the obviousness rejection on grounds that Skolnick teaches away from the Applicants' claims. Since Skolnick undermines that the claimed compounds have any reasonable expectation of success in treating depression, the obviousness rejection should be withdrawn (*see* M.P.E.P. 2142).

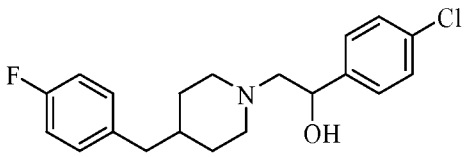
An obviousness rejection may be rebutted by showing that the art, in any material respect, teaches away from the claimed invention (*see* § M.P.E.P. 2144.05(III) (*citing In re Geisler*, 116 F.3d 1465, 1471 (Fed. Cir. 1997)). In making this showing, it has been required that a prior art reference must be considered in its entirety, *i.e.*, as a whole, including portions that would lead away from the claimed invention (*see* § M.P.E.P. 2141.02 (*citing W.L. Gore and Associates v. Garlock, Inc.*, 721 F.2d 1540 (Fed. Cir. 1983) *cert. denied*, 469 U.S. 851 (1984)).

Since the Examiner relied only on the abstract of Skolnick, Applicants attach with this response a copy of the entire article as Exhibit A for its complete consideration. A review of the entire article shows that the reference teaches away from the claimed invention for the following reasons. First, while Skolnick teaches that the NMDA receptor is involved in depression, the use of voltage dependent channel blockers would be undesirable because that class of NMDA antagonists induce psychotomimetic side effects. Second, Skolnick teaches that it is unpredictable whether NMDA antagonists will effectively treat depression.

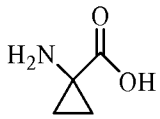
While Skolnick appears to teach that a physiological link exists between depression and NMDA receptors, the clinical application of this teaching is admitted by the reference to be uncertain. As is well-known to those skilled in the art, compounds with psychotomimetic properties induce hallucinations and other undesirable side effects. It is undesirable to have a therapeutic agent to treat depression that also induces psychotomimetic symptoms such as hallucinations. Skolnick teaches that the class of compounds called voltage dependent channel blockers, which antagonize the NMDA receptor, induce psychotomimetic activity. Because voltage dependent channel blockers cause such undesired side effects, including psychotomimetic activity, Skolnick teaches away from the use of this class of NMDA antagonists for the treatment of depression.

Skolnick's teaching away from the claimed invention is found as the article reviews several behavioral and neurochemical studies. The article states, "when taken together with the slowly developing nature of these phenomena [receptor adaptive alteration], strongly suggest

that adaptive changes in NMDA receptors are a final common pathway of AD [antidepressant] action" (*see* page 23, column 2, lines 14-19). Despite this assertion, the article admits, "[w]hile the therapeutic implications of these findings are obvious, applying this knowledge to the clinic may be problematic, since prototypic NMDA antagonists (*i.e.* voltage dependent channel blockers and competitive antagonists) possess psychotomimetic properties" (*see* page 24, lines 28-33, *citing* Sveinbjornsdottir et al., 1993, Muir et al., 1994, Krystal et al., 1994). This statement shows how Skolnick teaches away from using the claimed compounds, which are within the class of NMDA antagonists referred to as voltage dependent channel blockers. Skolnick does mention two NMDA antagonists which apparently lack psychotomimetic activity: eliprodil (α-(4-chlorophenyl)-4-[(4-fluorophenyl)methyl]-1-piperidineethanol) having the chemical formula:



and ACPC (1-aminocyclopropanecarboxylic acid) having the chemical formula:



However, these two compounds do not bear chemical structure similarity to the claimed compounds. Therefore, Skolnick's mention of eliprodil and ACPC does not suggests the use of the *claimed compounds* to treat depression.

In addition to teaching away from the therapeutic class of voltage dependent channel blockers, Skolnick also highlights the unpredictable nature of this entire class of NMDA antagonists to treat depression. After reviewing findings of neurochemical studies (downregulation of cortical β-adrenoceptors and radioligand binding to NMDA receptors) that may suggest that the NMDA receptor is involved in depression, the article aptly points out:

> "[t]he lack of stoichiometery between these measures [IC_{50} of glycine to inhibit [^{3}H]5,7DCKA binding and [^{3}H]CGP 39653 binding] raises the possibility that chronic [antidepressant] treatments *may produce multiple, discrete effects at NMDA receptors, or may exert differential effects at NMDA receptor subtypes*. The relative contribution or importance of these effects to either the onset of antidepressant action or efficacy *are unknown*, but raise important questions for future study."

See page 25, first full paragraph, *emphasis added*. Thus, even if a compound is a known NMDA antagonist, the affect, if any, of any such compound on depression is unclear – it may reduce or it may increase depression. Furthermore, the particular selection of an NMDA antagonist may affect the NMDA receptor in an unpredictable way, depending upon which NMDA receptor site or sites (there are at multiple sites, *see* Frontiers in Bioscience 3, e70-80, May 11, 1998) the antagonist interacts with. Thus, Skolnick teaches that it is uncertain whether NMDA antagonists will treat depression, and is only able to assert that the NDMA receptor "is involved" in the physiology of depression (*See* Skolnick abstract, last sentence). This teaching fails to support the Office Action's asserted conclusion that NMDA antagonists treat depression.

Because Skolnick teaches away from using voltage channel blockers for treating depression and teaches that the effect of NMDA antagonists on depression is unpredictable, Skolnick actually supports the nonobviousness of the instant claims. Accordingly, the rejection should be withdrawn.

B. Applicants provide evidence to show unexpected results/properties of the claimed compounds.

Applicants also provide herewith additional evidence showing that the claimed compounds for treating depression have a side effect profile in preclinical studies which is distinct from that of potent open channel blockers such as MK-801, which would not have been expected by one skilled in the art. Because one skilled in the art would have expected the recited compounds to have undesirable psychotomimetic activity, their use for treating depression is nonobvious.

Evidence submitted by an applicant of unexpected properties or surprising results can rebut an obviousness rejection (*see* M.P.E.P. 2144.08). Rebuttal evidence may show that the claimed invention yields unexpected or improved properties or properties not present in the art or a showing that the claimed compound possesses unexpected properties (*id.*) or the absence of an expected property (M.P.E.P. 716.02(b)(IV) (*citing Ex parte Mead Johnson & Co.*, 227 USPQ 78 (Bd. Pat. App. & Inter. 1985))). A showing of unexpected results for a single member of a claimed subgenus can rebut a *prima facie* case of obviousness (*id. citing In re Clemens*, 622 F.2d 1029, 1036 (CCPA 1980)). The evidence may also show that an unmet need exists (*see* M.P.E.P. 2144.08(II)(B) (*citing Graham v. John Deere Co.*, 383 U.S. 1, 17 (1966)).

Applicants provide the following evidence to show the unexpected nature of the claimed methods for treating depression. First, the Skolnick article identifies an unmet need in the art, which is satisfied by the Applicants' invention. As indicated in section A of this reply, Skolnick indicates that NMDA antagonists, specifically voltage channel blockers, have undesirable psychotomimetic side effects. This unmet need is corroborated in an article entitled, "NPS 1506, A Novel NMDA Receptor Antagonist and Neuroprotectant," MUELLER et al., Ann. N.Y. Acad. Sci., 890 (1), p. 450-457 (Exhibit B). Like the teachings of Skolnick, this article states,

> "the development of NMDA receptor antagonists has been hindered by an unfavorable side effect profile characterized by phencylidine (PCP)-like psychotomimetic effects, impairment of cognition, and a direct neurotoxic effect termed neuronal vacuolization."

See page 450. Thus, the art recognizes that an unmet need in the art exists, *i.e.*, a need to develop NMDA antagonists including voltage dependent channel blockers to treat depression that do not cause psychotomimetic side effects (*see* Skolnick, page 24, first column, second full paragraph).

The unmet need is satisfied by the Applicants' invention. The Mueller Academy of Science article demonstrates that NPS 1506 (Compound 60 as identified in the specification) does not induce psychotomimetic activity (*see* page 453, last paragraph – 454, and Figure 3). The Mueller article summarizes experiments measuring PCP-responses and showing that Compound 60 exhibits PCP-like effects only at doses above PCP and MK-801, another NMDA antagonist. In view of Skolnick's teaching that NMDA antagonists (and particularly voltage channel blockers) have psychotomimetic activity, the absence of this undesirable side effect was unexpected at the time of the invention. The absence of an undesirable property expected in the art shows that the claimed methods provide an unexpected and nonobvious quality.

Second, Applicants have discovered that the claimed compounds lack psychotomimetic activity and have a better safety profile than existing NDMA antagonists. The attached evidence also shows that compound 60 lacks other undesirable side effects. Compound 60 does not elicit MK-801-like behaviors (head weaving, backwards shuffling) even though MK-801 and Compound 60 are both NMDA antagonists (*see* Mueller Academy of Science article page 453, second to last paragraph). Compound 60 also does not impair cognition, as demonstrated by spatial learning in the Morris water maze test (*see* Figure 4 and page 455, first paragraph).

Compound 60 does not elicit neuronal vacuolization, even though other NMDA receptor antagonists MK-801 and CNS-1102 do elicit such effects (*see* Table 2 and page 455 second paragraph). These experimental comparisons demonstrate that the inventive methods treat depression with improved safety profiles unexpected for NMDA antagonists. The Applicants' claimed genus and species methods are therefore nonobvious.

The Office Action Does Not Provide Grounds For Rejecting Claims 25-29

The Office Action Summary indicates that claims 5,6 and 21-29 are rejected. Yet, in the Detailed Action, there are no grounds given for rejection of claims 25-29. The Detailed Action at point 4 applies a § 103 rejection to claims 5-6 and 21-24 but not to claims 25-29. Therefore, the Office Action does not provide grounds for rejecting claims 25-29.

Claim 25 recites the limitation "the compound is active at a serotonin reuptake site and at a N-methyl-D-aspartate (NMDA) receptor." This limitation has not been addressed by the Office Action. Accordingly, the claim should be allowed.

Claim 26 recites the limitation " the compound has an NMDA receptor IC_{50} of about 50 nM to about 1 μM." This limitation has not been addressed by the Office Action. Accordingly, the claim should be allowed.

Claim 27 recites the limitation "the compound has an NMDA receptor IC_{50} of about 100 nM to about 800 nM." This limitation has not been addressed by the Office Action. Accordingly, the claim should be allowed.

Claims 28 and 29 have not been addressed by the Office Action. Accordingly, these claims should be allowed.

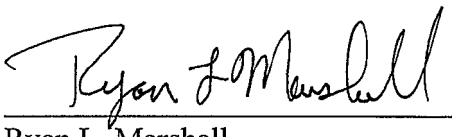
CONCLUSION

In summary, Applicants traverse the obviousness rejection on grounds that the cited art teaches away from using voltage dependent channel blockers. Applicants have also provided evidence of unexpected properties and results using the claimed methods.

In view of the above arguments and evidence, Claims 5-6 and 21-35 are believed to be in condition for allowance, and an early notice thereof is respectfully solicited. Should the Examiner determine that additional issues remain which might be resolved by a telephone conference, he is respectfully invited to contact Applicants' undersigned attorney.

In view of the above arguments and evidence, Claims 5-6 and 21-35 are believed to be in condition for allowance, and an early notice thereof is respectfully solicited. Should the Examiner determine that additional issues remain which might be resolved by a telephone conference, he is respectfully invited to contact Applicants' undersigned attorney.

Respectfully submitted,

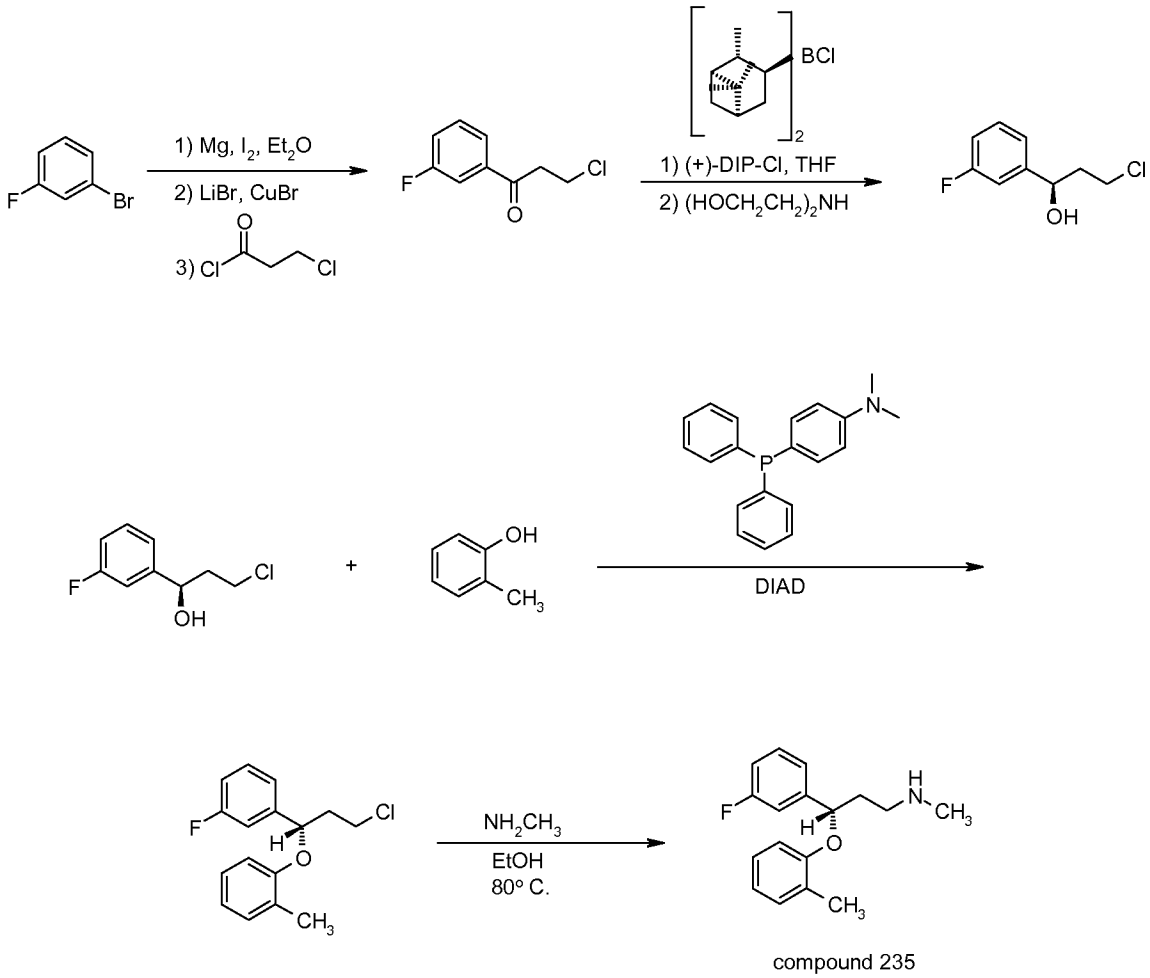


Ryan L. Marshall
Reg. No. 47,770
HOLLAND & HART LLP
P.O. Box 11583
Salt Lake City, UT 84147-0583
Telephone: (801) 799-5865
Fax: (801) 799-5700

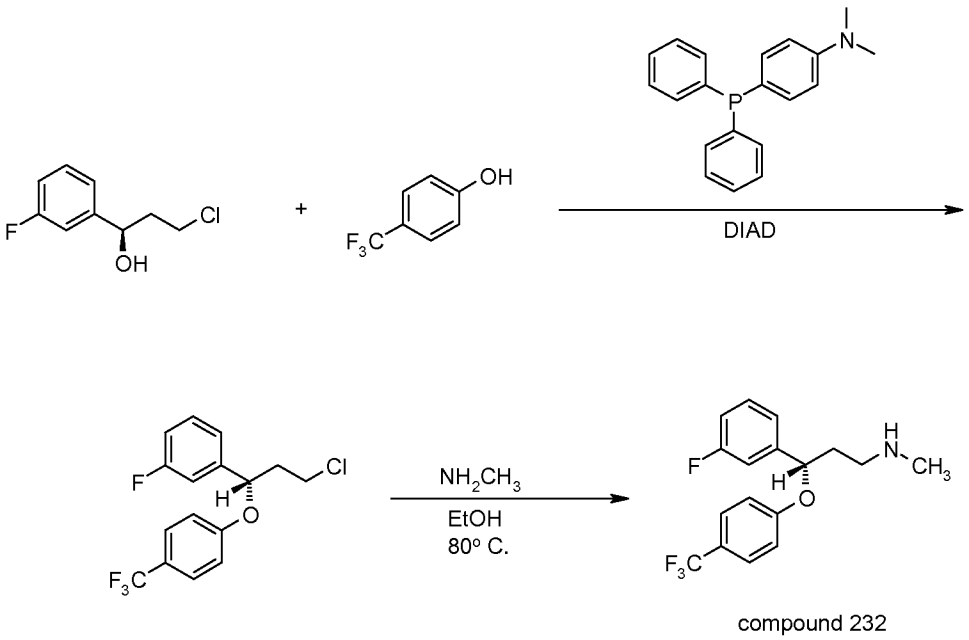
Date: June 27, 2006
RLM/jml
Enclosures: Replacement Pages 68-68A and 72-72A
Exhibit A – Skolnick article
Exhibit B – Mueller article

In a Parr flask the chloride prepared above (1.2 g, 4.54 mmol) was dissolved in ethanol (50 mL). Ammonium hydroxide (40 mL) was added and the reaction mixture was placed in an automatic Parr apparatus for 18 h at 90 °C. At the end of 18 h the solution was evaporated under vacuum. The oil was then transferred to a separatory funnel and washed with 0.1 M NaOH (2 × 25 mL) and water (2 × 25 mL), dried Na_2SO_4, filtered, and evaporated under vacuum. To a solution of resulting amine (0.703 g) in ethyl acetate (25 mL) was added a solution of maleic acid (0.309 g, 2.66 mmol) in ethyl acetate (5 mL). Hexane (10 mL) was added and the solution stirred until a solid was formed. The resulting white solid was filtered and dried in a vacuum oven to yield 0.600 g of product.

Synthesis of Compound 235



Compound 235 was synthesized in a four-step reaction sequence starting from commercially available materials. The Grignard reagent from 3-bromofluorobenzene was reacted with chloropropionyl chloride in the presence of copper bromide and lithium bromide to provide the chlorofluoropropiophenone. Following a method reported in the literature by Srebnik, M., Ramachandran, P. V., and Brown, H. C. (J. Org. Chem., 1988, 53, 2916-2920), the carbonyl group was stereoselectively reduced using (+)-*B*-chlorodiisoinocampheylborane. The resulting enantiomeric alcohol was then converted with stereochemical



compound 232

Compound 232 was synthesized in a four-step reaction sequence starting from commercially available materials. In a similar pathway as compound 235 was prepared, the Grignard reagent from 3-bromofluorobenzene was reacted with chloropropionyl chloride in the presence of copper bromide and lithium bromide to provide the chlorofluoropropiophenone. Following a method reported in the literature by Srebnik, M., Ramachandran, P. V., and Brown, H. C. (J. Org. Chem. 1988, 53, 2916-2920), the carbonyl group was stereoselectively reduced using (+)-*B*-chlorodiisopropylcampheylborane. The resulting enantiomeric alcohol was then converted with stereochemical inversion to its phenolic ether. The chloride functionality was then reacted with methylamine to provide the final product.

(*S*)-1-(3-Chloro-1-(3-fluorophenyl)propoxy)-4-trifluoromethylbenzene. To a solution of 4-(dimethylamino)phenyldiphenylphosphine (1.98 g, 6.49 mmol) in THF (40 mL) was added 3′-chloro-3-flourophenylpropanol (1.02 g, 5.41 mmol) followed by *para*-trifluoromethylphenol (1.14 g, 7.03 mmol). The reaction mixture was then placed in an ice bath and stirred for 10 min. Then, diisopropyl azodicarboxylate (DIAD, 1.28 mL 6.49 mmol) was added dropwise over a period of 1 min. The ice bath was then removed and the reaction mixture was stirred at room temperature for 4 h. The reaction mixture was then poured into diethyl ether (100 mL) and washed with 1 M NaOH (2 × 25 mL), 1 M HCl (2 × 25 mL), and brine (25 mL). The organic

layer was dried (anh. Na_2SO_4) and evaporated to provide 2.82 g of an oil which crystallized on standing. This material was

EXHIBIT A

Adaptation of N-Methyl-D-Aspartate (NMDA) Receptors following Antidepressant Treatment: Implications for the Pharmacotherapy of Depression

P. Skolnick[1], *R. T. Layer*[1], *P. Popik*[2], *G. Nowak*[2,3], *I. A. Paul*[3], *R. Trullas*[4]

[1] Laboratory of Neuroscience, NIDDK, National Institutes of Health, Bethesda, USA
[2] Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland
[3] Department of Psychiatry and Human Behavior, University of Mississippi Medical Center, Jackson, USA
[4] Neurobiology Unit, Department Bioanalytical Medicine, CSIC, Barcelona, Spain

NMDA antagonists mimic the effects of clinically effective antidepressants in both preclinical tests predictive of antidepressant action and procedures designed to model aspects of depressive symptomatology. These findings led to experiments demonstrating that chronic administration of NMDA antagonists to rodents results in a downregulation of cortical β-adrenoceptors, a phenomenon also observed following chronic treatment with many antidepressants. These neurochemical and behavioral similarities between antidepressants and NMDA antagonists prompted us to examine the impact of chronic antidepressant treatment on NMDA receptors. Chronic (14 days) but not acute (1 day) administration of seventeen different antidepressants to mice produced adaptive changes in radioligand binding to NMDA receptors. Detailed studies with three antidepressants (imipramine, citalopram, and electroconvulsive shock) show that these changes develop slowly, persist for some time after cessation of treatment, and (for imipramine and citalopram) are dose dependent. Moreover, following chronic treatment with imipramine, these changes in radioligand binding to NMDA receptors appear restricted to the cerebral cortex. Based on the consistency of these effects across antidepressant treatments, we propose that adaptive changes in NMDA receptors may be the final common pathway for antidepressant action. The recent demonstration (*Nowak* et al., 1995) that radioligand binding to NMDA receptors is altered in frontal cortex of suicide victims (compared to age and post-mortem interval matched controls) is consistent with the hypothesis (*Trullas* and *Skolnick*, 1990) that this family of ligand gated ion channels is involved in the pathophysiology of depression.

Introduction

Antidepressant (AD) therapy is remarkably diverse, encompassing both drugs and non-pharmacological interventions such as electroconvulsive shock (ECS). The observation that chronic administration is an apparent requirement for clinical improvement across AD treatments has led to the widely held assumption that an adaptation of neuronal circuitry is integral to the therapeutic response. The identification of a common adaptive mechanism resulting from chronic AD treatments has been a central theme of research in biological psychiatry for more than two decades. While chronic application of AD to animals can produce a variety of changes in the central nervous system, such effects (e. g. β-adrenoceptor downregulation) had not been uniformly observed across all AD treatments (reviewed in *Layer* et al., 1995 a). These observations, when taken together with the variety of in vitro actions produced by AD, could lead to the conclusion that an AD action may be effected through multiple pathways without invoking a common adaptive process. The obvious alternative to this thesis is that an adaptive process common to all AD has not been identified.

This paper will summarize evidence obtained during the past five years demonstrating that N-methyl-D-aspartate (NMDA) receptors are adaptively altered in circumscribed areas of the central nervous system following chronic AD treatment. The consistency of these neurochemical changes following AD drawn from every principal therapeutic class (including ECS), when taken together with the slowly developing nature of these phenomena, strongly suggest that adaptive changes in NMDA receptors are a final common pathway of AD action.

Moreover, the recent demonstration of alterations in radioligand binding to NMDA receptors in the frontal cortex of suicide victims (*Nowak* et al., 1995 a) is consistent with the hypothesis (*Trullas* and *Kolnick*, 1990) that this family of ligand gated ion channels is linked to the pathophysiology of depression.

Behavioral Studies Demonstrate the AD-Like Actions of Functional NMDA Antagonists

In our initial studies (*Trullas* and *Skolnick*, 1990), a competitive NMDA antagonist (2-amino-7-phosphonoheptanoic acid; AP-7), a voltage dependent channel blocker (dizocilpine; MK-801), and a partial agonist at strychnine-insensitive glycine sites (1-aminocyclopropanecarboxylic acid; ACPC) were found to reduce immobility in a forced swim test (FST) adapted for mice (*Porsolt* et al., 1977). While procedures such as the FST (*Porsolt* et al., 1977) are not generally considered animal models of depression, they are commonly used as preclinical predictors of AD action (*Porsolt* et al., 1981; *Steru* et al., 1987). Subsequent studies have demonstrated the AD-like actions of other competitive NMDA antagonists (e.g. CGP 397849 and CGP 39551) (*Maj* et al., 1992), the voltage dependent channel blocker memantine (*Moryl* et al., 1993), and eliprodil, an NMDA antagonist acting through polyamine coupled sites (*Layer* et al., 1995 b). Based on these findings, it appears that the ability of NMDA

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antagonists to mimic AD in the FST is neither strain nor species specific. *Panconi* et al. (1993) concluded that the motor stimulant properties of dizocilpine may result in a false positive in screening procedures such as the FST. However, the AD-like actions of NMDA antagonists which either have no remarkable effect or depress motor activity that locomotor stimulation per se cannot account for this mimicry across all classes of NMDA antagonists. The effects of ACPC in the FST amply illustrate this point. Thus, the dose dependent reduction in immobility produced by ACPC reaches a plateau at about the same level as maximally effective doses of imipramine. Although ACPC also produces a modest but statistically significant increase in open field activity (at doses 2–4 fold greater than a minimum effective dose in the FST [*Trullas* and *Skolnick*, 1990]), motor stimulants which produce false positives in the FST reduce immobility to values approaching zero.

Functional NMDA antagonists are also active in the chronic, mild stress paradigm developed by *Willner* et al. (1992) to model the anhedonia characteristic of depressive symptomatology. In this model, rats typically display a reduced sucrose consumption following ≥3 weeks of exposure to chronic mild stressors (CMS). Chronic treatment with AD will reverse CMS-induced reductions in sucrose consumption (*Muscat* et al., 1992). *Papp* and *Moryl* (1993, 1994) reported that dizocilpine and competitive NMDA antagonists (CGP 40116 and CGP 37849) were as effective as imipramine in restoring sucrose consumption.

While the therapeutic implications of these findings are obvious, applying this knowledge to the clinic may be problematic, since prototypic NMDA antagonists (i.e. voltage dependent channel blockers and competitive antagonists) possess psychotomimetic properties (*Sveinbjornsdottir* et al., 1993; *Muir* et al., 1994; *Krystal* et al., 1994). However, it appears that not all functional NMDA antagonists may share this liability since neither eliprodil (*Patat* et al., 1994) nor ACPC (Cherkofsky, 1995) have been reported to produce the psychotomimetic-like actions. A Phase II protocol has been designed for ACPC in depression (*R. Meibach*, personal communication).

Neurochemical Studies

Chronic treatment with functional NMDA antagonists: downregulation of cortical β-adrenoceptors

Chronic, but not acute treatment of laboratory animals with many clinically effective AD reduces the maximum number of cortical β-adrenoceptors (*Banerjee* et al., 1977; *Heninger* and *Charney*, 1987). While the relationship of β-adrenoceptor downregulation to both the mechanism of AD action and the pathophysiology of depression remains obscure, this phenomenon has been among the most consistent findings across AD therapies (*Heninger* and *Charney*, 1987). The ability of functional NMDA antagonists to mimic AD in behavioral paradigms led us to examine the effects of chronic treatment with such compounds on radioligand binding to cortical β-adrenoceptors. In our initial studies (*Paul* et al., 1992), mice were treated with ACPC or dizocilpine for 7 days, and [^{3}H]dihydroalprenolol (DHA) binding to β-adrenoceptors was measured in cerebral cortex. Chronic ACPC and dizocilpine reduced [^{3}H]DHA binding by 19% and 21%, respectively compared to a 23% reduction elicited by a similar course of imipramine. *Klimek* and *Papp* (1994) reported a similar reduction in [^{3}H]DHA binding following chronic dizocilpine administration to rats. These investigators also demonstrated that like imipramine, dizocilpine blocked the increase in β-adrenoceptors produced by chronic, mild stressors. In toto, these findings are consistent with the behavioral studies detailed in the previous section, and support the hypothesis that functional NMDA receptor antagonists possess AD properties.

Chronic AD treatments effect adaptive changes in radioligand binding to NMDA receptors

In our initial studies (*Nowak* et al., 1993), mice were injected daily for two weeks with imipramine. Parallel groups received two weeks of saline or a single injection of imipramine or saline. Radioligand binding to NMDA receptors, strychnine-insensitive glycine receptors, and NMDA receptor coupled cation channels were evaluated using [^{3}H]CGP 39653, [^{3}H]5,7-dichlorokynurenic acid (DCKA) and [^{3}H]dizocilpine, respectively. Chronic, but not acute treatment with imipramine significantly altered radioligand binding to NMDA receptors. The two alterations which appear common to all AD examined can be summarized as follows: 1. A reduction (compared to vehicle treated mice) in the potency of glycine to inhibit [^{3}H]5,7-DCKA binding to strychnine-insensitive glycine receptors. In the case of imipramine, this decrease in potency was ~2.5-fold; and 2. A reduction in the proportion of high affinity glycine sites inhibiting [^{3}H]CGP 39653 binding to NMDA receptors. This reduction was ~28% following 14 days of imipramine treatment. Similar effects were not apparent in mice administered a single dose of imipramine. Moreover, these effects were observed in cerebral cortex but not in hippocampus, striatum or basal forebrain. A followup study demonstrated that a two week regimen of imipramine (as well as ECS) produced similar changes in radioligand binding to NMDA receptors in rat cortex (*Paul* et al., 1993), indicating these effects were not species specific.

If these effects on radioligand binding to NMDA receptors produced by imipramine are adaptive, then they should develop slowly and persist for some time after cessation of treatment. Consistent with this hypothesis, time course studies revealed the first statistically significant change in the IC_{50} of glycine to inhibit [^{3}H]5,7-DCKA binding to mouse cortex required 14 days of treatment with imipramine, 10 days with citalopram, and 7 days with ECS, respectively. The effect of imipramine and citalopram were dose dependent in this measure. Moreover, the effect of imipramine was not fully manifested in this measure following 14 days of treatment, since a 21 day regimen further the IC_{50} of glycine to 330% of control (*Paul* et al., 1994).

If adaptive changes in NMDA receptors represent a final common pathway of AD action, then such changes should be manifest after chronic treatment with a wide variety of AD, regardless of chemical structure or in vitro actions. In order to test this hypothesis, mice were chronically injected with seventeen AD and the potency of glycine to inhibit [^{3}H]5,7-DCKA binding and/or the effect of glycine on [^{3}H]CGP 39653 binding measured in cortical tissues (*Paul* et al., 1994; *Layer* et al., 1995a; *Nowak* et al., 1995b). The seventeen AD used in these studies have demonstrated efficacy in controlled, double blind trials and include tricyclic AD, monoamine oxidase inhibitors,

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the so-called "atypical" agents, and ECS. Chronic treatment with all the AD tested produced changes in one or both of the neurochemical measures examined (*Paul* et al., 1994; *Layer* et al. 1995a). A limited number of substances not generally considered as AD were also examined for their effects on ligand binding to NMDA receptors. Chronic treatment with these drugs (e.g. salbutamol, chlordiazepoxide, D-deprenyl) failed to effect similar changes. At a minimum, the ability of chronic AD treatment to produce adaptive changes in radioligand binding to NMDA receptors is a more robust predictor of clinical efficacy than either changes in β-adrenoceptor density or efficacy in the FST (*Layer* et al., 1995a). These latter measures are perhaps the most widely used biochemical and behavioral predictors of AD activity, respectively.

These neurochemical studies failed to demonstrate a clearcut stoichiometric relationship between AD-induced changes in the IC_{50} of glycine to inhibit [^{3}H]5,7DCKA binding and reductions in the proportion of high affinity glycine sites inhibiting [^{3}H]CGP 39653 binding to NMDA receptors. This is perhaps best illustrated by the effects of chronic treatment with citalopram. Thus, a 14 day regimen of citalopram increased the IC_{50} of [^{3}H]5,7DCKA ~2-fold, but appeared to abolish the high affinity component of glycine displaceable [^{3}H]CGP 39653 binding (*Paul* et al., 1994; *Layer* et al., 1995a). A subsequent study (*Nowak* et al., 1995b) confirmed this remarkable difference in the actions of citalopram, underscoring the dramatic effect of this AD on [^{3}H]CGP 39653 binding. In vehicle treated mice, glycine displacement of [^{3}H]CGP 39653 is best fit to a 2 site model, with high affinity displacement representing ~ 60 –70% of the total pool. In this study, glycine inhibition of [^{3}H]CPG 39653 binding was best fit to a 2 site model in 6/6 subjects, while chronic citalopram treatment resulted in curves which were best fit to a 1 site (low affinity) model in 5/6 mice. In contrast, this study revealed only a ~ 50% increase in the IC_{50} of glycine to inhibit [^{3}H]5,7-DCKA binding (*Nowak* et al., 1995b). Moreover, consistent with our previous study, these changes were manifested in cortical but not hippocampal membranes. The lack of stoichiometry between these measures raises the possibility that chronic AD treatments may produce multiple, discrete effects at NMDA receptors, or may exert differential effects at NMDA receptor subtypes. The relative contribution or importance of these effects to either the onset of AD action or efficacy are unknown, but raise important questions for future study.

Conclusions

The molecular mechanisms responsible for AD-induced changes in radioligand binding to NMDA receptors are unknown, and are currently under investigation in our laboratories. Nonetheless, the neurochemical data summarized in this paper represents the description of a consistent, selective adaptation of a neurotransmitter-associated receptor produced by chronic treatment with all major classes of AD. Based on the AD-like actions of functional NMDA antagonists, we hypothesized (*Trullas* und *Skolnick*, 1990) that NMDA receptors might also be involved in the pathophysiology of depression. Despite the widespread distribution of NMDA receptors in the central nervous system, the effects of imipramine and citalopram indicate that adaptive changes in radioligand binding following imipramine and citalopram are restricted to cerebral cortex (*Nowak* et al., 1993; 1995b). *Moghaddam* (1993) has reported a remarkable increase in glutamate and aspartate release in prefrontal cortex (relative to other brain regions) following either swim or restraint stress. In view of the association between repeated stress and the development of depression (*Willner* et al., 1992), this observation may provide a bridge linking our findings to a potential role for NMDA receptors in depressive disorders. The recent demonstration (*Nowak* et al., 1995a) of changes in radioligand binding to NMDA receptors in frontal cortex of suicide victims provides an independent line of evidence linking this family of ligand-gated ion channels to the pathophysiology of depression.

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Dr. *P. Skolnick*
Laboratory of Neuroscience
NIDDK, NIH
Building 8, Room 111
Bethesda, MD 20892-0008
USA

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EXHIBIT B

NPS 1506, A Novel NMDA Receptor Antagonist and Neuroprotectant

Review of Preclinical and Clinical Studies

ALAN L. MUELLER,[a] LINDA D. ARTMAN, MANUEL F. BALANDRIN, ELLEN BRADY, YONGWEI (ERIC) CHIEN, ERIC G. DELMAR, KAREN GEORGE, ALLISON KIERSTEAD, THOMAS B. MARRIOTT, SCOTT T. MOE, MICHAEL K. NEWMAN, JOANNA L. RASZKIEWICZ, ELIZABETH L. SANGUINETTI, BRADFORD C. VAN WAGENEN, AND DAVID WELLS

NPS Pharmaceuticals, Inc., Salt Lake City, Utah, USA

ABSTRACT: NPS 1506 is a moderate affinity, uncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist. NPS 1506 is neuroprotective in rodent models of ischemic stroke, hemorrhagic stroke, and head trauma, with a 2-hr window of opportunity. Neuroprotectant doses of NPS 1506 ranged from approximately 0.1–1.0 mg/kg, with peak plasma concentrations ranging from 8–80 ng/mL. Even at doses producing behavioral toxicity, NPS 1506 did not elicit MK-801-like behaviors, did not generalize to phencyclidine (PCP), and did not elicit neuronal vacuolization.

In a Phase I study, intravenous (i.v.) doses of NPS 1506 from 5–100 mg were well tolerated and provided plasma concentrations in excess of those required for neuroprotection in rodents. Adverse events at the 100-mg dose included mild dizziness and lightheadedness, and mild to moderate ataxia. Neither PCP-like psychotomimetic effects nor cardiovascular effects were noted. The long plasma half-life of NPS 1506 (~60 hr) suggests that a single i.v. dose will provide prolonged neuroprotection in humans.

INTRODUCTION

The *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptor is an attractive target in the search for neuroprotective agents. Competitive and noncompetitive antagonists of NMDA receptors prevent neuronal cell death in various *in vivo* animal models of stroke and head trauma.[1,2] However, the development of NMDA receptor antagonists has been hindered by an unfavorable side effect profile characterized by phencyclidine (PCP)-like psychotomimetic effects, impairment of cognition, and a direct neurotoxic effect termed neuronal vacuolization.[3,4]

NPS 1506 was synthesized as part of an NMDA receptor antagonist medicinal chemistry program based on polyamine-containing spider toxins and diphenylpropylamine-type antihistaminergics and anticholinergics. The compound 3,3-diphenylpropylamine (3,3-DPPA) was discovered in a small-scale screening effort as the

[a]Corresponding author: Alan L. Mueller, Ph.D., NPS Pharmaceuticals, Inc., Salt Lake City, UT 84108. Phone, 801/583-4939; fax, 801/583-4961.
e-mail, amueller@npsp.com

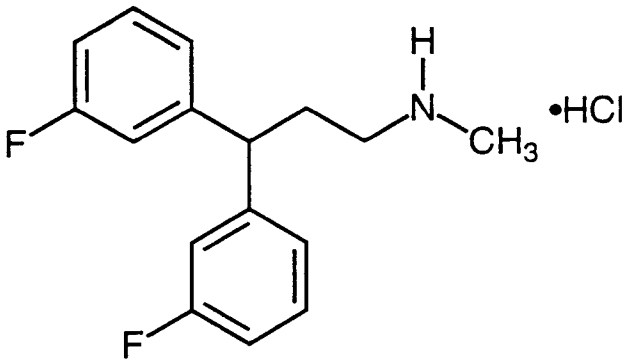

FIGURE 1. Chemical structure of NPS 1506 (hydrochloride salt).

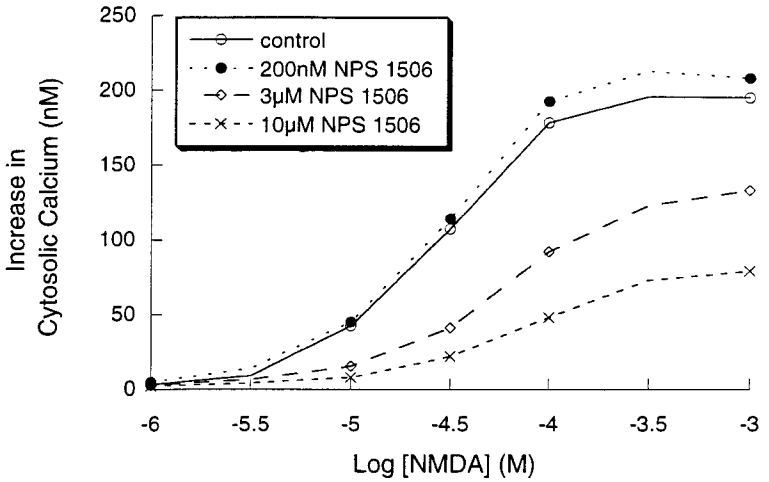

FIGURE 2. Nature of the block of NMDA-induced increases in cytosolic calcium in cultured RCGCs by NPS 1506. The concentration-response curve to NMDA (in the presence of a fixed concentration of 1 μM glycine) was shifted rightward and downward by the addition of increasing concentrations of NPS 1506, indicative of noncompetitive blockade.

initial lead compound possessing moderate affinity for the NMDA receptor.[5] This pharmacophore was rapidly optimized to provide analogs with varying potencies as NMDA receptor antagonists.[5,6] Here we briefly summarize the preclinical profile of NPS 1506, and report our initial Phase I clinical data.

PRECLINICAL PHARMACOLOGY OF NPS 1506

NPS 1506 (FIG. 1) produced a concentration-dependent inhibition of NMDA/glycine-induced increases in cytoplasmic calcium in cultured rat cerebellar granule

TABLE 1. Summary of preclinical neuroprotectant efficacy of NPS 1506

Study	Dosing Regimen	Degree of Neuroprotection	Comments
Temporary focal ischemia (rat MCAO suture model[7]; 2-hr period of ischemia plus 46-hr period of reperfusion)	0.1, 0.3, or 1.0 mg/kg i.v. administered 2 and 6 hr post-occlusion (dose expressed as HCl salt)	32%, 44%, and 48% reduction in infarct volume, respectively	1. 2-hr window of opportunity. 2. Double-dose paradigm more robust and consistent than single-dose paradigm. 3. Neuroprotectant plasma concentrations range from 8–80 ng/mL (C_{max}).
Temporary focal ischemia (rat MCAO suture model[7]; 2-hr period of ischemia plus 46-hr period of reperfusion)	Bolus loading dose plus continuous 8-hr infusion to maintain plasma concentrations of 20 or 80 ng/mL	43% and 57% reduction in infarct volume, respectively	1. 2-hr window of opportunity. 2. Robust and consistent neuroprotection.
Permanent focal ischemia (rat MCAO suture model[7]; 24-hr period of ischemia)	0.3 or 1.0 mg/kg i.v. administered 30 min and 4 hr post-occlusion (dose expressed as free base)	0% and 45% reduction in infarct volume, respectively	1. 1.0 mg/kg dose provided significant neuroprotection in 3 out of 4 separate, independent studies.
Permanent focal ischemia (rat MCAO suture model[7]; 24-hr period of ischemia)	Bolus loading dose plus continuous 24-hr infusion to maintain plasma concentration of 80 ng/mL	No significant neuroprotectant effect	1. Absence of neuroprotectant activity noted in 2 separate, independent studies.
Hemorrhagic stroke (rat; intrastriatal bacterial collagenase model[8])	1.0 mg/kg i.p. administered 1 hr post-collagenase injection (dose expressed as HCl salt)	Significant improvement in brain potassium and sodium content	1. Neuroprotection absent when treatment delayed 4 hours.
Closed head trauma (rat; weight drop method[9])	1.0 mg/kg i.v. administered 1 and 4 hr post-injury (dose expressed as HCl salt)	Significant improvement in brain electrolytes, edema and neurological severity score	1. No behavioral toxicity noted with NPS 1506.
Traumatic brain injury (rat; lateral fluid-percussion model[10])	1.0 mg/kg i.v. administered 0.25 and 4 hr post-injury (dose expressed as free base)	Significant improvement in memory score and CA3 neuron survival	1. No behavioral toxicity noted with NPS 1506.

cells (RCGCs), with an IC_{50} of 476 nM. Inhibition of [^{3}H]MK-801 binding to rat cortical membranes was observed with similar concentrations of NPS 1506 (IC_{50} =

664 nM). Studies in RCGCs demonstrate that the blockade produced by NPS 1506 is noncompetitive with respect to agonist (FIG. 2), and uncompetitive, i.e., the magnitude of block at a single concentration of NPS 1506 increases as the concentration of agonist is increased (data not shown).

Electrophysiological experiments using NMDA receptor subunits recombinantly expressed in *Xenopus* oocytes demonstrate that the block produced by NPS 1506 is both use- and voltage-dependent, consistent with open-channel block. NPS 1506 was equipotent at blocking the various NR2 subunits coexpressed with NR1A, and does not demonstrate subunit selectivity.

NPS 1506 is neuroprotective *in vivo*, as demonstrated in a variety of rodent models (summarized in TABLE 1). In a rat model of temporary focal ischemic stroke, namely, middle cerebral artery occlusion (MCAO) induced by the suture method, the dose of NPS 1506 required for significant neuroprotection ranged from approximately 0.1–1.0 mg/kg. These doses were most effective when administered twice, approximately 3–4 hr apart. Peak plasma concentrations following neuroprotectant dosing regimens were between 8–80 ng/mL. More robust and consistent neuroprotection was provided when NPS 1506 was administered as a bolus loading dose followed by a constant infusion in order to maintain plasma concentrations over an 8-hr period. In this temporary focal ischemia model, NPS 1506 demonstrated a 2-hr window of opportunity.

NPS 1506 was neuroprotective in a rat permanent MCAO occlusion model when administered as two 1-mg/kg intravenous (i.v.) injections, 3–5 hr apart (TABLE 1). This neuroprotectant effect was lost when NPS 1506 was administered as a bolus loading dose followed by a 24-hr constant infusion.

NPS 1506 was neuroprotective in two rat models of traumatic brain injury when administered as two 1-mg/kg bolus i.v. injections, 3–4 hr apart (TABLE 1). The maximum plasma concentrations of NPS 1506 in these studies were estimated to be approximately 80 ng/mL. Lower doses of NPS 1506 were not examined in these models of head injury.

NPS 1506 provided modest neuroprotection in a rat model of intracerebral hemorrhagic stroke, namely, the intrastriatal bacterial collagenase model, when administered as a single 1-mg/kg intraperitoneal (i.p.) dose (TABLE 1). This neuroprotection occurred when NPS 1506 was administered 1 hr after the injection of collagenase, prior to complete hematoma formation. Importantly, NPS 1506 did not worsen outcome in this model of hemorrhagic stroke.

At doses greater than those required for neuroprotection, NPS 1506 did not elicit in rodents the characteristic side effect profile that is typical of potent, noncompetitive open-channel antagonists, such as MK-801. MK-801-like behaviors, such as head weaving or backwards shuffling, were not noted. Rather, the most consistent sign of behavioral toxicity of NPS 1506 in rodents was a whole body tremor that was dose-dependent in terms of onset, severity and duration. This tremor was consistently observed when plasma concentrations of NPS 1506 exceeded 400 ng/mL (see below).

NPS 1506 was evaluated for PCP-like discriminative stimulus effects in rats trained to discriminate PCP from saline. NPS 1506 had no PCP-like effects at doses between 1–5 mg/kg i.p. (FIG. 3). The 5-mg/kg i.p. dose produced a maximum mean 34% PCP-lever response, but this dose was associated with a 40% decrease in overall

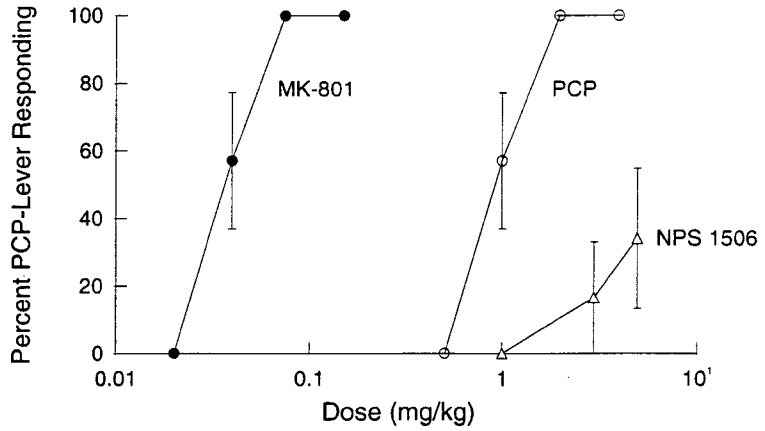

FIGURE 3. Lack of generalization to PCP by NPS 1506. MK-801 potently and dose-dependently substituted for PCP in rats pretrained to discriminate PCP from saline. NPS 1506 had no PCP-like effects at doses between 1–5 mg/kg i.p. (as the HCl salt). The 5-mg/kg i.p. dose produced a maximum mean 34% PCP-lever response, but this dose was associated with a 40% decrease in overall response rate (not shown). Data are expressed as mean ± SEM.

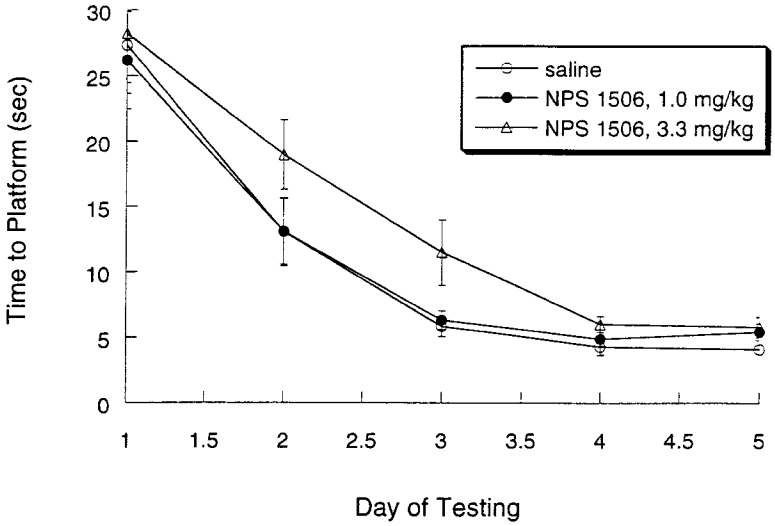

FIGURE 4. Lack of long-lasting impairment of spatial learning by NPS 1506. NPS 1506 (1 mg/kg i.v. as the free base) did not alter normal spatial learning in the Morris water maze. A dose of 3.3 mg/kg i.v. caused a significant slowing of spatial learning. However, performance in the group treated with 3.3 mg/kg NPS 1506 was not different from that in the saline-treated control animals by the final day of training. Data are expressed as mean ± SEM.

response rate. MK-801 fully substituted for PCP at doses of 0.075 and 0.15 mg/kg i.p. that had no effect on overall response rates.

The effects of NPS 1506 on spatial learning in the Morris water maze were examined and compared to those of MK-801. NPS 1506 (1 mg/kg i.v. 20 min before the start of daily training trials) did not alter normal spatial learning in the Morris water maze (FIG. 4). An NPS 1506 dose of 3.3 mg/kg i.v. caused a significant slowing of spatial learning. However, performance in the group treated with 3.3 mg/kg NPS 1506 was not different from that in the saline-treated control animals by the final day of training. In addition, probe trial scores, which demonstrate memory for the platform location, were not different between groups. Thus, at the doses tested, NPS 1506 did not cause a permanent impairment of spatial learning and memory. In contrast, MK-801 (0.1–0.3 mg/kg i.p.) disrupted spatial learning in the Morris water maze task. Importantly, the 0.1-mg/kg dose of MK-801 did not impair performance in a visible platform training task, strongly suggesting that the spatial learning deficit observed at this dose was due to an effect on the memory system itself and not due to sensory or motor impairment.

NPS 1506 was examined for its ability to induce neuronal vacuolization in neurons of rat cingulate and retrosplenial cortices. NPS 1506 (20 mg/kg i.p. or 10 mg/kg i.v.) did not elicit neuronal vacuolization (TABLE 2). In contrast, neuronal vacuolization was produced by the NMDA receptor antagonists, MK-801 (5 mg/kg i.p.) and CNS-1102 (20 or 30 mg/kg i.p.). These results were confirmed and extended in a Good Laboratory Practice (GLP) compliant study using NPS 1506 doses ranging from 1–10 mg/kg i.v. (data not shown).

PRECLINICAL TOXICOLOGY OF NPS 1506

NPS 1506 was examined in a series of GLP-compliant i.v. toxicology studies ranging from single-dose studies to 14-day studies. NPS 1506 was administered i.v. to rats and dogs either as a slow (60-sec) bolus or by continuous infusion. Additionally, the effects of NPS 1506 on the gastrointestinal, renal, cardiovascular, and pulmonary systems were investigated in a series of ancillary pharmacology studies.

The predominant, consistent findings in the single-dose, 60-sec bolus and 24-hr infusion i.v. studies in rats and dogs involved transient central nervous system (CNS)-related events that consisted of whole body tremors (convulsions at high doses), hypertonia, salivation, emesis, hypothermia (rats), hyperpyrexia (dogs), vocalization, and death. The severity and duration of these effects were dose-related but independent of the dosing regimen. No histologic evaluations were performed in these studies.

Repeated bolus-dose studies and continuous infusion studies in rats and dogs have been completed for 7 and 14 days. As in the single-dose studies, the consistent, early systemic finding in these studies was the appearance of tremors and, less frequently, seizures or convulsions, in both species. Other dose-related effects seen in rats were inflammation/necrotizing inflammation at the injection site, hematologic response to inflammation, hypertonia and vocalization. Other than the noted irritation/inflammation at the injection site, there was no histopathological evidence of target-organ toxicity elicited by NPS 1506 in any of these studies. Rats appeared to tolerate the infusion procedure somewhat better than the bolus administration. Salivation, emesis, vocalization, hyperpyrexia, and rigidity were noted in dogs at the higher doses tested.

TABLE 2. Summary of neuronal vacuolization data

Treatment	Incidence of Vacuolization
MK-801 (5 mg/kg i.p.)	vacuoles present in 6 out of 7 rats
CNS-1102 (20 mg/kg i.p.)	vacuoles present in 1 out of 2 rats
CNS-1102 (30 mg/kg i.p.)	vacuoles present in 1 out of 1 rats
NPS 1506 (20 mg/kg i.p.)	vacuoles present in 0 out of 1 rats
NPS 1506 (10 mg/kg i.v.)	vacuoles present in 0 out of 4 rats
Water or saline controls	vacuoles present in 0 out of 3 rats

The no observed adverse effect level (NOAEL) for systemic toxicity in the 7- and 14-day repeated bolus-dose studies in rats was 4.5 and 3.0 mg/kg/day, respectively. In dogs, the NOAEL for systemic toxicity was 2 mg/kg/day for both the 7- and 14-day repeated bolus-dose regimens. Following bolus administration to rats or dogs, the first signs of systemic toxicity were associated with NPS 1506 plasma concentrations greater than 400 ng/mL.

The NOAEL for systemic toxicity in the 7- and 14-day continuous infusion studies in rats was 10 mg/kg/day. In dogs, the NOAEL for systemic toxicity was 3.0 mg/kg/day for both the 7- and 14-day continuous infusion studies. Consistent with the findings from the 7- and 14-day bolus dose studies, observations of systemic toxicity were associated with NPS 1506 plasma concentrations greater than 400 ng/mL.

NPS 1506 was also evaluated in other GLP-compliant toxicology studies. NPS 1506 did not cause hemolysis in human, dog, or rat blood at final concentrations of 50 μg/mL and below. Frank hemolysis was evident at final concentrations greater than 50 μg/mL, which is significantly higher than the maximum plasma concentrations expected in clinical trials. NPS 1506 was not mutagenic in an Ames assay, mouse lymphoma cell assay, or an *in vivo* mouse micronucleus assay. In addition, NPS 1506 did not cause delayed-type hypersensitivity in mice, did not cause protein flocculation in human, dog, or rat serum or plasma, and had no effect on platelet aggregation in whole blood at concentrations up to 30 μg/mL.

PHASE I CLINICAL STUDY OF NPS 1506

The initial Phase I study of NPS 1506 was a double-blind, placebo-controlled, ascending-dose tolerability and pharmacokinetic study in healthy male volunteers (age 18–40, weight 60–90 kg). NPS 1506 was administered as a single i.v. infusion at rates ranging from 1.0–2.67 mg/min. Doses of NPS 1506 from 5–100 mg were well tolerated and provided plasma concentrations in excess of those required for neuroprotection in rodents. Adverse events noted at the 100-mg dose included mild dizziness and lightheadedness, and mild to moderate ataxia. Importantly, neither PCP-like psychotomimetic effects nor cardiovascular effects were noted. NPS 1506 has a very large volume of distribution at steady-state (V_{ss}) of approximately 17 L/kg, consistent with extensive distribution throughout the body. The long terminal plasma half-life (~60 hr) suggests that a single i.v. dose is sufficient to provide prolonged neuroprotection.

SUMMARY AND CONCLUSIONS

NPS 1506 is a moderate-affinity, uncompetitive NMDA receptor antagonist. The compound is neuroprotective in a variety of animal models of stroke and head injury, and has a side effect profile in preclinical studies distinct from that of potent open-channel blockers such as MK-801. NPS 1506 is well tolerated in man at doses (plasma concentrations) shown to be neuroprotective in rodents. The clinical development of NPS 1506 as an acute use neuroprotectant is ongoing.

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